

What is claimed is:

1. 1. A method for detecting a GPCR-binding partner complex comprising a GPCR and a GPCR binding partner, said method comprising:
  3. (a) culturing a cell producing a first and a second polypeptide, wherein at least one of said first and said second polypeptides is a GPCR,
  5. (b) lysing said cell;
  6. (c) contacting said first polypeptide with a substrate having affinity for said first polypeptide, under conditions suitable for binding of said first polypeptide to said substrate; and
  8. (d) detecting the presence of said second polypeptide on said substrate, wherein said detecting is direct;
10. wherein the presence of said second polypeptide on said substrate is indicative of a GPCR-binding partner complex.
1. 2. The method of claim 1, wherein said first polypeptide is a GPCR and said second polypeptide is a GPCR binding partner..
1. 3. The method of claim 1, wherein said first polypeptide is a GPCR binding partner and said second polypeptide is a GPCR.
1. 4. The method of claim 1, wherein at least one of said first and said second polypeptides is an orphan GPCR.
1. 5. The method of claim 1, wherein at least one of said first and second polypeptides is a native GPCR.
1. 6. The method of claim 1, wherein at least one of said first and said second polypeptides is a constitutively activated GPCR.
1. 7. The method of claim 1, wherein both of said first and said second polypeptides are GPCRs.
1. 8. The method of claim 7, wherein said GPCRs are the same GPCR.
1. 9. The method of claim 7, wherein said GPCRs are different GPCRs.

- 1 10. The method of claim 1, wherein one of said first and said second polypeptides is not a GPCR.
- 1 11. The method of claim 10, wherein said one of said first and said second polypeptide is a G-  
2 protein.
- 1 12. The method of claim 1, wherein at least one of said first and said second polypeptides is  
2 recombinant.
- 1 13. The method of claim 1, wherein said first polypeptide is bound to said substrate using an  
2 antibody that specifically binds to said polypeptide.
- 1 14. The method of claim 1, wherein said first and second polypeptides are different and wherein  
2 detection of said second polypeptide comprises binding of an antibody specific for said second  
3 polypeptide.
- 1 15. The method of claim 1, wherein said first polypeptide comprises an affinity tag, and said  
2 isolating step comprises binding of the affinity tag to the affinity substrate.
- 1 16. The method of claim 15 wherein said affinity tag is an epitope tag.
- 1 17. The method of claim 1, wherein said second polypeptide is detectably labeled, and said  
2 detecting step comprises detecting said detectable label.
- 1 18. The method of claim 17, wherein said label is an epitope tag and said detecting comprises  
2 binding of a fluorochrome-conjugated antibody specific for said epitope tag.
- 1 19. The method of claim 17, wherein said polypeptide is fused to a reporter protein.
- 1 20. The method of claim 19, wherein said reporter protein is luciferase.
- 1 21. The method of claim 19, wherein said first polypeptide is detectably labeled, and said detecting  
2 step is by FRET.

- 1    22.    The method of claim 1, wherein said method further comprises contacting said cell with a
- 2    cross-linking agent prior to said lysing step (b).
- 1    23.    The method of claim 1, wherein said first and said second polypeptides are endogenously co-expressed in at least one cell type, tissue, or tissue sub-region.
- 1    24.    The method of any one of claims 1-22, wherein said method further comprises selecting prior  
2    to said culturing step (a) said first and said second polypeptides wherein said first and said second  
3    polypeptides are coexpressed in a least one cell type, tissue or tissue sub-region.
- 1    25.    A method for detecting a GPCR-binding partner complex comprising a GPCR and a GPCR  
2    binding partner, said method comprising:
  - 3        (a) culturing a cell producing a first and a second polypeptide, wherein at least one of said first  
4    and said second polypeptides is a GPCR,
  - 5        (b) lysing said cell;
  - 6        (c) contacting said first polypeptide with an addressable substrate having affinity for said first  
7    polypeptide, under conditions suitable for binding of said first polypeptide to said substrate in an  
8    addressable manner; and
  - 9        (d) detecting the presence of said second polypeptide on said substrate, wherein said detecting  
10   is direct;  
11   wherein the presence of said second polypeptide on said substrate is indicative of a GPCR-binding  
12   partner complex.
- 1    26.    The method of claim 25, wherein said first polypeptide is a GPCR and said second polypeptide  
2    is a GPCR binding partner.
- 1    27.    The method of claim 25, wherein said first polypeptide is a GPCR binding partner and said  
2    second polypeptide is a GPCR.
- 1    28.    The method of claim 25, wherein at least one of said first and said second polypeptides is an  
2    orphan GPCR.
- 1    29.    The method of claim 25, wherein at least one of said first and said second polypeptides is a  
2    native GPCR.

- 1    30.     The method of claim 25, wherein at least one of said first and said second polypeptides is a  
2     constitutively activated GPCR.
- 1    31.     The method of claim 25, wherein both of said first and said second polypeptides are GPCRs.
- 1    32.     The method of claim 31, wherein said GPCRs are the same GPCR.
- 1    33.     The method of claim 31, wherein said GPCRs are different GPCRs.
- 1    34.     The method of claim 25, wherein one of said first and said second polypeptides is not a GPCR.
- 1    35.     The method of claim 34, wherein said one of said first and said second polypeptide is a G-  
2     protein.
- 1    36.     The method of claim 25, wherein at least one of said first and said second polypeptides is  
2     recombinant.
- 1    37.     The method of claim 25, wherein said method further comprises contacting said cell with a  
2     cross-linking agent prior to said lysing step (b).
- 1    38.     The method according to any one of claims 25-37 wherein said addressable affinity substrate is  
2     spatially addressable.
- 1    39.     The method of claim 38 wherein said spatially addressable affinity substrate is a 96-well or  
2     384-well format affinity substrate.
- 1    40.     The method of claim 38, wherein said first polypeptide is bound to said substrate using an  
2     antibody that specifically binds to said first polypeptide.
- 1    41.     The method of claim 38, wherein said first and said second polypeptides are different and  
2     wherein detection of said second polypeptide comprises binding of an antibody specific for said second  
3     polypeptide.

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1    42.    The method of claim 38, wherein said first polypeptide comprises an affinity tag, and said  
2    isolating step comprises binding of the affinity tag to the affinity substrate.

1    43.    The method of claim 42 wherein said affinity tag is an epitope tag.

1    44.    The method of claim 38, wherein said second polypeptide is detectably labeled, and said  
2    detecting step comprises detecting said detectable label.

1    45.    The method of claim 44, wherein said polypeptide is fused to a reporter protein.

1    46.    The method of claim 45, wherein said reporter protein is luciferase.

1    47.    The method of claim 45, wherein said first polypeptide is detectably labeled, and said detecting  
2    step is by FRET.

1    48.    The method according to any one of claims 25-37 wherein said addressable affinity substrate is  
2    spectrophotometrically addressable.

1    49.    The method of claim 48, wherein wherein said first polypeptide is bound to said substrate using  
2    an antibody that specifically binds to said polypeptide.

1    50.    The method of claim 48, wherein said second polypeptide comprises an epitope tag, and said  
2    detecting step comprises detection of the epitope tag.

1    51.    The method of claim 50 wherein said detection comprises binding of a fluorochrome-  
2    conjugated antibody specific for the epitope tag.

1    52.    The method of claim 38 or claim 48, wherein said first and said second polypeptides are  
2    endogenously co-expressed in at least one cell type, tissue, or tissue sub-region.

1    53.    The method of any one of claims 25-51, wherein said method further comprises selecting prior  
2    to said culturing step (a) said first and said second polypeptides wherein said first and said second  
3    polypeptides are coexpressed in a least one cell type, tissue or tissue sub-region.

1       54.     A method of identifying whether a candidate polypeptide is a binding partner for a GPCR,  
2     comprising the step of detecting a GPCR-binding partner complex comprising said candidate  
3     polypeptide and said GPCR according to the method of any one of claims 1-53, wherein detection of  
4     said complex is indicative of said candidate polypeptide being a binding partner of said GPCR.

1       55.     A method for detecting a GPCR-binding partner complex, said method comprising:  
2              (a) culturing a plurality of cells, each cell producing a first and a second polypeptide, wherein  
3     at least one of said first and said second polypeptides is a GPCR, and wherein each cell produces a  
4     different GPCR;  
5              (b) lysing said cells;  
6              (c) contacting said first polypeptide from each cell with an addressable substrate having  
7     affinity for said first polypeptide, under conditions suitable for binding of said first polypeptide to said  
8     substrate at an address specific for said cell; and  
9              (d) detecting the presence of said second polypeptide on said substrate, wherein said detecting  
10    is direct;  
11    wherein the presence of said second polypeptide at an address on said substrate is indicative of a  
12    GPCR-binding partner complex comprising said first polypeptide and said second polypeptides  
13    produced by the cell having said address.

1       56.     The method of claim 55 wherein said plurality of cells is at least 2, at least 5, at least 10, at  
2     least 15; at least 20, at least 25, at least 50, or at least 100 cells.

1       57.     The method of claim 55, wherein said first and said second polypeptides are endogenously co-  
2     expressed in at least one cell type, tissue, or tissue sub-region.

1       58.     The method of claim 55, wherein said method further comprises selecting prior to said  
2     culturing step (a) said first and said second polypeptides wherein said first and said second  
3     polypeptides are coexpressed in a least one cell type, tissue or tissue sub-region.

1       59.     A method for detecting a GPCR-binding partner complex, said method comprising:  
2              (a) culturing a cell, said cell producing a first and a plurality of a second polypeptide, wherein  
3     each of said plurality of said second polypeptide is different and wherein at least one of said first and  
4     said plurality of said second polypeptide is a GPCR;  
5              (b) lysing said cell;

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6                   (c) contacting said first polypeptide with a substrate having affinity for said first polypeptide,  
7 under conditions suitable for binding of said first polypeptide to said substrate; and

8                   (d) detecting the presence of said second polypeptide on said substrate, wherein said detecting  
9 is direct;  
10 wherein the presence of said second polypeptide on said substrate is indicative of at least one GPCR-  
11 binding partner complex comprising said first polypeptide and said second polypeptide.

1       60.       The method of claim 59 wherein said GPCR-binding partner complex is detected, further  
2 comprising repeating steps (a) to (d) one or more times with subsets of said plurality of said second  
3 polypeptide, said subsets encompassing said plurality, until a GPCR-binding partner complex is  
4 detected from at least one cell producing a said first polypeptide and a single said second polypeptide.

1       61.       The method of claim 59 wherein said plurality of said second polypeptides is at least 2, at least  
2       5, at least 10, at least 15, at least 20, at least 25 said, at least 50, or at least 100 of second polypeptide.

1       62.       The method of claim 59 wherein said producing of a first and a plurality of a second  
2 polypeptide is transient.

1       63.       The method of claim 59, wherein said first and second polypeptides are endogenously co-  
2 expressed in at least one cell type, tissue, or tissue sub-region.

1       64.       The method of claim 59, wherein said method further comprises selecting prior to said  
2 culturing step (a) said first and said second polypeptides wherein said first and said second  
3 polypeptides are coexpressed in a least one cell type, tissue or tissue sub-region.

1       65.       The method of any one of claims 55-64, wherein said first polypeptide is a GPCR and said  
2 second polypeptide is a GPCR binding partner.

1       66.       The method of any one of claims 54-64, wherein said first polypeptide is a GPCR binding  
2 partner and said second polypeptide is a GPCR.

1       67.       The method of any one of claims 55-64, wherein at least one of said first and said second  
2 polypeptides is an orphan GPCR.

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1    68.    The method of any one of claims 55-64, wherein at least one of said first and said second  
2    polypeptide is a native GPCR.

1    69.    The method of any one of claims 55-64, wherein at least one of said first and said second  
2    polypeptides is a constitutively activated GPCR.

1    70.    The method of any one of claims 55-64, wherein both of said first and said second  
2    polypeptides are GPCRs.

1    71.    The method of claim 70, wherein said GPCRs are the same GPCR.

1    72.    The method of claim 70, wherein said GPCRs are different GPCRs.

1    73.    The method of any one of claims 55-64, wherein one of said first and said second polypeptides  
2    is not a GPCR.

1    74.    The method of claim 73, wherein said one of said first and said second polypeptide is a G-  
2    protein.

1    75.    The method of any one of claims 55-64, wherein at least one of said first and second  
2    polypeptides is recombinant.

1    76.    The method of any one of claims 55-64, wherein said first polypeptide is bound to said  
2    substrate using an antibody that specifically binds to said polypeptide.

1    77.    The method of any one of claims 55-64, wherein said first and second polypeptides are  
2    different and wherein detection of said second polypeptide comprises binding of antibody specific for  
3    said second polypeptide.

1    78.    The method of any one of claims 55-64, wherein said first polypeptide comprises an affinity  
2    tag, and said isolating step comprises binding of the affinity tag to the affinity substrate.

1    79.    The method of claim 78 wherein said affinity tag is an epitope tag.

1    80.    The method of any one of claims 55-64, wherein said second polypeptide is detectably labeled,  
2    and said detecting step comprises detecting said detectable label.

1    81.    The method of claim 80, wherein said label is an epitope tag and said detecting comprises  
2    binding of a fluorochrome-conjugated antibody specific for said epitope tag.

1    82.    The method of claim 80, wherein said polypeptide is fused to a reporter protein.

1    83.    The method of claim 82, wherein said reporter protein is luciferase.

1    84.    The method of claim 82, wherein said first polypeptide is detectably labeled, and said detecting  
2    step is by FRET.

1    85.    The method of any one of claims 55-64, wherein said method further comprises contacting said  
2    cell with a cross-linking agent prior to said lysing step (b).

1    86.    A method for identifying whether a candidate agent modulates binding of a GPCR to a binding  
2    partner for the GPCR, said method comprising the step of determining whether there is a difference in  
3    the amount of a GPCR-binding partner complex comprising said GPCR and said binding partner  
4    detected according to a method of any one of claims 1-52, wherein said difference is determined for  
5    contacting or not contacting said candidate agent with said first and said second polypeptides prior to  
6    said detecting step (d) of said method and wherein an alteration in said amount of said second  
7    polypeptide detected on said affinity substrate is indicative of an agent that modulates binding of said  
8    GPCR to said binding partner for the GPCR.

1    87.    The method of claim 86, wherein said first and said second polypeptides are contacted with  
2    said candidate agent during step (a), step (b) or step (c).

1    88.    The method of claim 86, wherein said candidate agent is a small molecule, a peptide, a ligand  
2    for said GPCR, or an antibody.

1    89.    The method of claim 86, wherein said modulator reduces said binding of a GPCR to a binding  
2    partner for the GPCR by more than about 10%, more than about 20%, more than about 30%, more than  
3    about 40%, or more than about 50% as compared to said binding in the absence of said agent.

1    90.    The method of claim 86, wherein said modulator increases said binding of a GPCR to a  
2    binding partner for the GPCR by more than about 10%, more than about 25%, more than about 50%,  
3    more than about 100%, more than about 200%, more than about 300%, more than about 400%, or more  
4    than about 500.

1    91.    The method according to any one of claims 1-90 wherein said first and said second  
2    polypeptides are both mammalian.

1    92.    The method according to claim 91 wherein said first and second polypeptides are both human.

1    93.    A composition comprising an addressable affinity substrate, said addressable affinity substrate  
2    comprising a plurality of addresses having affinity for different GPCRs.

1    94.    The composition of claim 93 wherein said addressable affinity substrate is spatially  
2    addressable.

1    95.    The composition of claim 94 wherein said spatially addressable affinity substrate comprises at  
2    least about 100, at least about 200, at least about 300, at least about 400, or at least about 500  
3    addresses.

1    96.    The composition of claim 93 wherein said addressable affinity substrate is  
2    spectrophotometrically addressable.

1    97.    The composition of claim 96 wherein said spectrophotometrically addressable affinity substrate  
2    comprises at least about 2, at least about 5, at least about 10, at least about 15, at least about 20, or at  
3    least about 25 addresses.

1    98.    A composition comprising a cell, said cell producing a first and a second polypeptide, wherein  
2    said first and said second polypeptides are a GPCR and a GPCR binding partner, and wherein said first  
3    polypeptide comprises an affinity tag and said second polypeptide is fused to a reporter protein.

1    99.    The composition of claim 98, wherein said first polypeptide is a GPCR and said second  
2    polypeptide is a GPCR binding partner.

- 1 100. The composition of claim 98, wherein said first polypeptide is a GPCR binding partner and  
2 said second polypeptide is a GPCR.
- 1 101. The composition of claim 98, wherein at least one of said first and said second polypeptides is  
2 an orphan GPCR.
- 1 102. The composition of claim 98, wherein at least one of said first and said second polypeptides is  
2 a native GPCR.
- 1 103. The composition of claim 98, wherein at least one of said first and said second polypeptides is  
2 a constitutively activated GPCR.
- 1 104. The composition of claim 98, wherein both of said first and said second polypeptides are  
2 GPCRs.
- 1 105. The composition of claim 104, wherein said GPCRs are the same GPCR.
- 1 106. The composition of claim 104, wherein said GPCRs are different GPCRs.
- 1 107. The composition of claim 98, wherein one of said first and said second polypeptides is not a  
2 GPCR.
- 1 108. The composition of claim 107, wherein said polypeptide is a G-protein.
- 1 109. The composition of claim 98, wherein at least one of said first and second polypeptides is  
2 recombinant.
- 1 110. The composition of claim 98 wherein said affinity tag is an epitope tag.
- 1 111. The composition of claim 98, wherein said reporter protein is luciferase.

1    112.   The composition of claim 98, wherein said GPCR and said GPCR binding partner of said first  
2    and said second polypeptides are endogenously co-expressed in at least one cell type, tissue, or tissue  
3    sub-region.

1    113.   The composition of any one of claims 98-111, wherein said GPCR and said GPCR binding  
2    partner of said first and said second polypeptides are selected prior to said producing for co-expression  
3    in a least one cell type, tissue or tissue sub-region.

1    114.   The composition of any one of claims 98-113 wherein said first and said second polypeptides  
2    are both mammalian.

1    115.   The composition of claim 114 wherein said first and said second polypeptides are both human.

1    116.   A composition comprising a library of GPCRs, wherein said library comprises a plurality of  
2    pairs of isolated polynucleotide wherein both a first and a second said polynucleotide of said pair  
3    comprises a first nucleotide sequence encoding the same GPCR, said encoded GPCR of one said pair  
4    being different from said encoded GPCR of any other said pair, and wherein said first polynucleotide  
5    of said pair further comprises a second nucleotide sequence encoding an in-frame affinity tag and said  
6    second polynucleotide of said pair further comprises a third nucleotide sequence encoding an in-frame  
7    reporter protein.

1    117..   The composition of claim 116 wherein said affinity tag is an epitope tag and said reporter  
2    protein is luciferase.

1    118.   The composition of claim 116 wherein said library comprises at least about 50 said pairs, at  
2    least about 100 said pairs, at least about 200 said pairs, at least about 300 said pairs, at least about 400  
3    said pairs, or at least about 500 said pairs.

1    119.   The composition of claim 116 wherein said encoded GPCR is native.

1    120.   The composition of claim 116 wherein said encoded GPCR is mammalian.

1    121.   The composition of claim 120 wherein said encoded GPCR is human.